

# HISTOLOGY OF THE REPRODUCTIVE SYSTEM OF THE SOFT-SHELL CLAM (*MYA ARENARIA*)

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In many kinds of experimental work with marine organisms, a means of inducing them to spawn under laboratory conditions is often essential. Although many species of lamellibranch molluscs spawn readily in response to suitable stimuli, the soft-shell clam (*Mya arenaria*) is more refractory, responding poorly and erratically to the commonly used techniques of stimulation. To gain a better understanding of the spawning mechanism in *Mya*, a detailed study of the morphology and histology of its reproductive system was undertaken. Although the anatomy of *Mya* was described in some detail by Vlès (1909), only a few lines were devoted to the reproductive organs. Coe and Turner (1938) described the reproductive system more thoroughly, but less attention was given to the accessory organs than to the gonad itself. Furthermore, the discussion of the system was presented from a developmental viewpoint and dealt primarily with immature animals. The purpose of the present paper is to describe the morphology and histology of the reproductive system as a whole, with particular emphasis on structures other than the gonad.

Because *Mya* differs from many other species in its responsiveness to artificial spawning stimuli, any peculiarities in the morphology of its reproductive system must be examined in the light of their possible relationship to spawning activities. For this reason, comparative studies were made of two additional species: the surf clam, *Spisula solidissima*, and the hard clam, *Mercenaria mercenaria*, to determine whether *Mya* differed significantly in the morphology of its reproductive system. Observations on the spawning activities of these species were also made to learn what aspects of function might correspond with peculiarities in structure.

## METHODS

The information presented in this paper was based on the following sources:

- (1) Several hundred histological preparations of the gonad tissue of *Mya arenaria*, representing all seasons of the year, were examined.
- (2) Gross dissections and examinations were made on fresh specimens of *Mya arenaria*, *Mercenaria mercenaria* and *Spisula solidissima*.
- (3) Living specimens of these three species were held in aquaria and their spawning activities observed.
- (4) Serial sections of tissues fixed in Bouin's fluid were made through the region of the genital papillae of 13 randomly selected specimens of *Mya*, 5 of *Spisula* and three of *Mercenaria*. These sections were stained with haematoxylin and eosin and mounted on slides.

Photographs were made of various structures in the histological preparations and reconstructions in the form of sketches were made to clarify the spatial relationships of the structures.

Spawning of the living specimens was artificially induced by a cyclic fluctuation in water temperature, or in a few cases by injecting 1 ml. of 0.1 N ammonium hydroxide into the gonad through the mantle strap.

## RESULTS

In general arrangement, the reproductive system of *Mya* is similar in both sexes. The gonads are paired organs consisting of highly ramified tubules bearing numerous terminal and lateral alveoli. In a well-nourished, ripe individual these alveoli are closely packed and fill much of the visceral mass. The tubules of each gonad, by a series of confluences, eventually merge into a pair of gonoducts (Fig. 1)

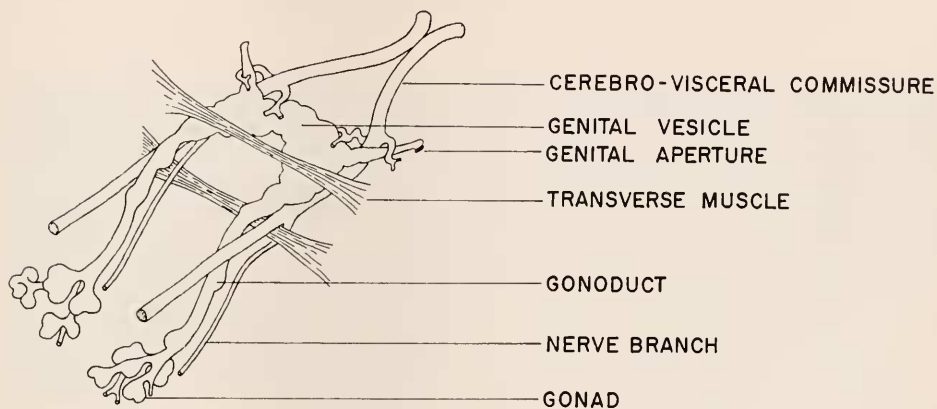


FIGURE 1. Semi-diagrammatic reconstruction of the reproductive system and associated structures in *Mya arenaria*.

which lead caudad and dorsad towards the region of the genital apertures. The apertures are situated on tiny papillae located on either side of the dorsal apex of the visceral mass (Fig. 2), just anterior to the foot retractor muscle. According to Vlès (1909), the gonads remain isolated from one another throughout the system, so that ink injected into one of the genital apertures will permeate the genital organ on one side of the clam only. While this mutual isolation seemed to be true in some of the individuals which I examined, in most specimens the gonoducts were joined in a common chamber or vesicle (Figs. 1 and 3), lying between and slightly anterior to the genital apertures. The vesicle is often bilobed, suggesting a tendency toward separation. Leading caudad and laterally from this vesicle are two short ducts (referred to henceforth as the "terminal gonoducts") which lead to the genital apertures. The apertures, opening into the dorsal pallial cavity, are in the form of slits curving from the termini to the lateral sides of the papillae.

The paired nerve trunks of the cerebro-visceral commissure pass directly under the terminal gonoducts, giving off many small branchlets which invest the con-

nective tissue in this region. A larger branch from each of these nerve trunks follows the gonoduct from the genital vesicle deep into the gonad itself. The wall of the visceral mass contains abundant muscular tissue; connecting these walls transversely are numerous bands of muscle fibers which pass through the gonad.

The histological structure of the gonad alveoli has been described by Coe and Turner (1938). These alveoli consist of a thin basement membrane or wall surrounding a group of large, transparent follicle cells, which may fill the alveolus solidly, or may separate in the center to form a lumen. The gametes arise from the basement membrane between the peripheral follicle cells. The gonoducts and their branches resemble the alveoli in histological structure, except that the large, transparent cells tend to be more elongate, oriented perpendicularly to the basement membrane, and a lumen is always present. Gametes are also proliferated from the basement membrane of the gonoducts, although less abundantly than in the alveoli. In some specimens the transparent cells lining the gonoduct are reduced in size and appear to be disintegrating. Where this process is taking place, clusters of clear droplets appear in the lumen, suggesting holocrine secretory activity.

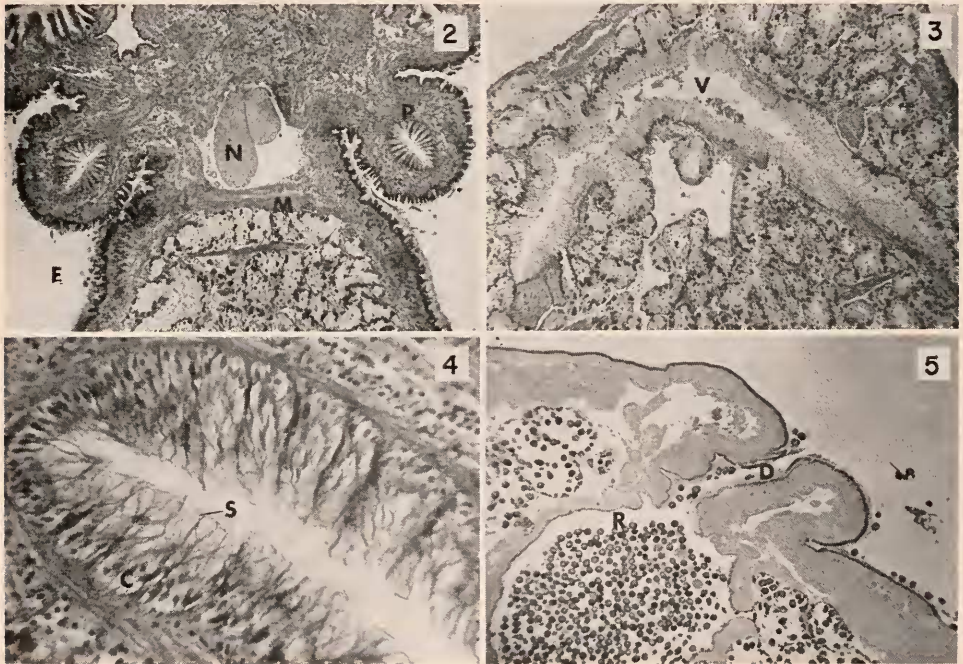


FIGURE 2. Cross-section through the visceral mass of *Mya arenaria* at the level of the genital papillae. Papillae (P), cerebro-visceral nerve commissures (N), transverse muscles (M) and dorsal pallial chamber (E) are indicated.  $\times 35$ .

FIGURE 3. Cross-section through the visceral mass of *Mya arenaria* at the level of the genital vesicle (V).  $\times 35$ .

FIGURE 4. Cross-section of terminal gonoduct of *Mya arenaria* taken through genital papilla, showing both ciliated columnar (C) and non-ciliated "secretory" cells (S).  $\times 300$ .

FIGURE 5. Longitudinal section through genital papilla of *Spisula solidissima*, showing terminal gonoduct (D) and enlarged portion of main gonoduct (R) used as reservoir for eggs.  $\times 25$ .

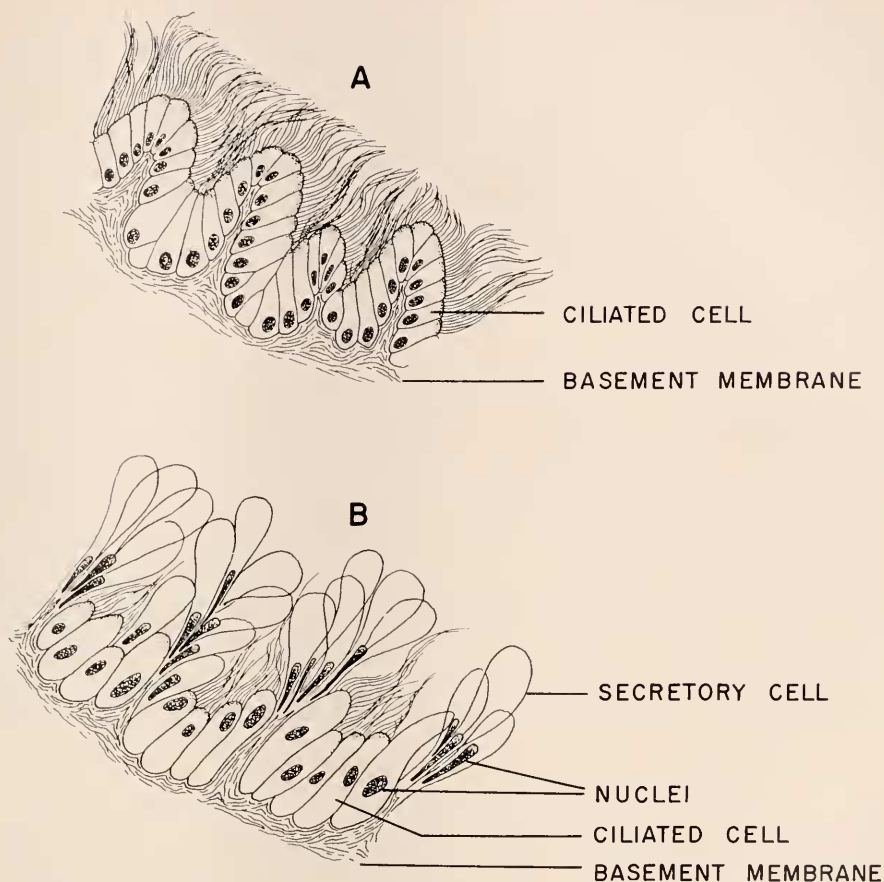


FIGURE 6. Camera lucida drawings, somewhat simplified for clarity, showing portions of the ciliated (A) and non-ciliated (B) epithelial tissues in the terminal gonoduct of *Mya arenaria*.

The tissue lining the terminal gonoducts deserves particular notice. The interior walls are wrinkled with longitudinal ridges of connective tissue upon which rests an epithelium of columnar and other cells, the nature and arrangement of which is variable. In the simplest case, all are ciliated columnar cells with prominent elliptical nuclei (Fig. 6A). The cytoplasm stains lightly with eosin, more intensely so near the ciliated ends of the cells. In a somewhat more complex arrangement, the longitudinal ridges may extend farther into the lumen to form thin folds rather than simple ridges. The ciliated columnar cells on these folds become or are replaced by elongated, non-ciliated cells with bulbous ends containing a granular cytoplasm. In what appears to be an advanced stage of this development, the bulbous ends become very prominent and more transparent, and groups of them on the crests and sides of the folds of connective tissue suggest, in section, clusters of fruit on a stalk (Figs. 4 and 6B). Groups of these specialized cells alternate with groups of ciliated cells, or may sometimes replace them entirely. Many

intermediate arrangements between the extremes of simple ciliated columnar epithelium to the highly modified bulbous cells can be found in different individuals. The arrangement may differ between the two terminal gonoducts of the same individual.

The function of the ciliated cells is undoubtedly to assist in the extrusion of gametes from the genital aperture. That of the other cells is uncertain, but their appearance suggests a secretory function. Because of the variation in the arrangement and nature of this epithelial tissue, it is possible that a cyclic transition occurs in a manner similar to that in tissues of certain reproductive organs in higher animals. However, no relationship was apparent between the condition of the gonoduct epithelium and the sex or the stage in the seasonal reproductive cycle of the clams examined.

The reproductive systems of both *Mercenaria*, described by Loosanoff (1937a, 1937b), and *Spisula* are similar in many respects to that of *Mya*. One essential point of difference is the lack in the former species of the large follicle cells characteristic of *Mya*. Also, both *Mercenaria* and *Spisula* possess more abundant muscular tissue throughout the visceral mass. The genital vesicle of *Mya*, described above, seems to be less well defined in *Mercenaria* or *Spisula*, but these species do have greatly enlarged portions of the gonoducts which occupy the same region.

The lining of the terminal gonoduct in *Mercenaria* is similar to that of *Mya*. On the other hand, that of *Spisula* is quite different. In this species the epithelium is a simple and relatively smooth layer of ciliated columnar cells, differing little from the ciliated epithelium on the exterior surface of the papillae (Fig. 5). Although some longitudinal folding is apparent, the prominent alternating ridges and furrows characteristic of *Mya* are absent.

#### DISCUSSION

There are certain aspects of observed spawning processes in clams which appear to be related to the morphology of the reproductive system and which suggest the function of some of the structures observed in histological preparations. One of these aspects is the relative slowness of response to artificial spawning stimuli characteristic of *Mya*. Not only does this species respond much less readily than either *Mercenaria* or *Spisula* at any time, but its response declines noticeably toward the latter part of the spawning season. In 1961, when the laboratory spawning experiments were conducted, the period during which *Mya* could be found in a predominantly ripe condition lasted from late May through July, and some ripe individuals could be found even in mid-August. It was chiefly during the month of June, however, when this species responded most readily to spawning stimuli. Even during this brief period, the frequency at which spawning could be induced declined steadily during the period that any group of clams was held under observation. After the first week in July, no responses could be obtained, either from clams already under observation or from freshly dug specimens, even though many of them were still apparently ripe. On one occasion a group of clams expelled large quantities of blood and tissue cells, indicating a mechanical spawning reflex in response to the stimuli, but shed no gametes.

Examination of several hundred sections of *Mya* gonad tissue, representing all seasons of the year, has shown that rarely were ripe ova strongly preponderant in

the ovaries. Usually, even in "ripe" individuals, eggs were present in all stages of development. It is difficult to understand, therefore, how a clam can emit several million eggs in a few minutes, as commonly occurs during spawning, if these must be freed directly from the ovaries.

What appears to be more likely is that eggs are accumulated before spawning in a reservoir from which they can be extruded *en masse*. This situation can be readily observed in both fresh and histologically prepared ripe specimens of both *Mercenaria* and *Spisula* (Fig. 5), where enormous numbers of ova (or spermatozoa) are accumulated in the enlarged portions of the gonoducts in the postero-dorsal region of the visceral mass. It seems evident that the genital vesicle of *Mya* is homologous to the enlarged oviducts of the other two species and serves as a reservoir for the accumulation of gametes prior to spawning. By exerting pressure with a finger on the visceral mass of a ripe *Mya* in the locality of this vesicle, one can force from some individuals a limited quantity of gametes through the genital apertures. Once this quantity has been extruded, further pressure produces no more gametes, even though the ovaries are still replete.

If ripe gametes are accumulated in a reservoir prior to spawning, one might expect to find them in serial sections through the gonoducts and genital vesicle. Although only three of the serially sectioned specimens were ripe, one of these did contain substantial numbers of accumulated ova in the genital vesicle. Nevertheless, such accumulation was far less prevalent or exuberant in *Mya* than in either *Mercenaria* or *Spisula*. This fact could account for the relatively poor response of *Mya* to spawning stimuli if an accumulation of ripe gametes in a reservoir is a necessary precedent to spawning.

An extension of this argument may also help explain why apparently "ripe" clams in June spawn more readily in the laboratory than apparently "ripe" clams in July or August. Apparent ripeness is merely a measure of the abundance of ripe gametes in the gonad at a single moment. It indicates nothing about the rate at which they are being proliferated; yet the frequency of occurrence of clams with full reservoirs must certainly be dependent on the rate of gamete production. If the gamete production rate declines later in the season, response to spawning stimuli should decline accordingly.

Comparative studies of the three species have revealed another relationship between spawning physiology and morphology which, although its significance is not clear, warrants attention. In every experiment where *Mya* was induced to spawn in the laboratory, the eggs were shed with the nucleus membrane dissolved, that is, in the initial stages of meiosis. In this respect, *Mercenaria* is similar to *Mya*. On the other hand, *Spisula* releases eggs with the nucleus intact, and the initiation of meiosis ("activation") occurs after the sperm has penetrated the egg (Allen, 1953; Costello *et al.*, 1957). This is true of other lamellibranchs, and the American oyster (*Crassostrea virginica*), although not included among the species which I examined, is a pertinent example.

Apparently related to these differences is the fact that the eggs of *Spisula* and *Crassostrea* can be excised artificially from the ripe ovary and readily fertilized by placing them in clean water with a little sperm, after which they will develop normally (Costello, *et al.*, 1957; Galtsoff, 1937). Viable eggs can seldom be obtained in this manner from either *Mercenaria* (Loosanoff, 1953) or *Mya*. *Mya*

and *Mercenaria*, the eggs of which are "activated" before spawning, and which cannot be fertilized successfully unless so "activated," both have the complex epithelium of the terminal gonoduct described earlier. *Spisula* and *Crassostrea*, which release their eggs before "activation," and from which viable eggs can be excised directly from the ovaries, have a relatively simple epithelium lining the gonoduct. This is shown for *Spisula* in Figure 7; Galtsoff (1961) shows a similar figure for *Crassostrea*.

Obviously, further investigation is required to clarify this relationship, and to determine the extent to which it applies to other species of lamellibranch.<sup>1</sup> Also, the examination of more individuals, collected in various stages of their seasonal reproductive cycle, should provide clues as to the role played by the epithelial cells in the terminal gonoduct.

#### SUMMARY

1. The reproductive organs of *Mya arenaria* consist of paired alveolar gonads, connected by paired gonoducts to a (usually) common vesicle. From the vesicle, a pair of short terminal gonoducts lead to the genital apertures.

2. The region occupied by the vesicle, terminal gonoducts and genital apertures is invested with muscular and connective tissue and is highly innervated with branchlets from the cerebro-visceral commissures which pass through the region. Paired branches from these nerve trunks also follow the main gonoducts into the gonad.

3. The terminal gonoducts are lined by longitudinally furrowed epithelium of ciliated columnar cells. In many individuals groups of these cells alternate with groups of non-ciliated, elongated cells with bulbous tips, apparently secretory in function.

4. Evidence is cited to show that sex products are accumulated in the genital vesicle prior to spawning. Homologous structures in two other species of lamellibranch are described which serve the same purpose.

5. An apparent relationship is shown between the time and site of egg activation and the nature of the epithelium of the terminal gonoduct of four species of lamellibranch molluscs.

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<sup>1</sup> Since the completion of this manuscript, I had occasion to examine sections through the genital papilla of the common mussel (*Mytilus edulis*) and found that the lining of the terminal gonoduct of this species is very similar to that of *Mya*. *Mytilus*, like *Mya*, also sheds its eggs after nuclear breakdown.

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